

Substitute Sequence Listing

<110> Hamoen, Leendert W
 Kuipers, Oscar Paul
 Lindner, Johanna Cordula
 <120> Novel Bacterial Expression System
 <130> I-2002.012 US
 <140> 10/523,729
 <141> 2003-08-01
 <150> PCT/EP2003/008506
 <151> 2003-08-01
 <150> EP 02078248.8
 <151> 2002-08-07
 <160> 51
 <170> PatentIn version 3.3
 <210> 1
 <211> 29
 <212> DNA
 <213> Bacillus subtilis
 <400> 1
 ttgtaaaggg acaagagctt tggataat 29
 <210> 2
 <211> 94
 <212> DNA
 <213> Bacillus subtilis
 <400> 2
 ttgtaaaggg acaagagctt tggataata taaaattgtg agtaatagaa ttattgctcc 60
 ttgcccatta tgggccgctt agtccaaaag gagg 94
 <210> 3
 <211> 113
 <212> DNA
 <213> Bacillus subtilis
 <400> 3
 gatgcagttg taaagggaca agagctttgg tataatataa aattgtgagt aatagaatta 60
 ttgctccttg cccattatgg gccgcttagt ccaaaaggag gtgcaaacag atg 113
 <210> 4
 <211> 20
 <212> DNA
 <213> Bacillus subtilis
 <220>
 <221> misc_feature
 <222> (8)..(8)

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<223> n is a, t, g or c

<220>

<221> misc_feature

<222> (13)..(14)

<223> n is a, t, g or c

<220>

<221> misc_feature

<222> (17)..(18)

<223> n is a, t, g or c

<220>

<221> misc_feature

<222> (20)..(20)

<223> n is a, t, g or c

<400> 4

atttaatatnta tannatnnan

20

<210> 5

<211> 33

<212> DNA

<213> Bacillus subtilis

<220>

<221> misc_feature

<222> (6)..(6)

<223> n is a, g, t or c

<220>

<221> misc_feature

<222> (11)..(13)

<223> n is a, g, t or c

<220>

<221> misc_feature

<222> (21)..(21)

<223> n is a, g, t or c

<220>

<221> misc_feature

<222> (26)..(27)

<223> n is a, g, t or c

<220>

<221> misc_feature

<222> (30)..(31)

<223> n is a, g, t or c

<220>

<221> misc_feature

<222> (33)..(33)

<223> n is a, g, t or c

<400> 5

ttattnattt nnnatttaatt ntatannatn nan

33

<210> 6

<211> 33

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<212> DNA
 <213> Bacillus subtilis

 <400> 6
 ttattcattt ccgatttaat gtataggatg cag 33

 <210> 7
 <211> 43
 <212> DNA
 <213> Bacillus subtilis

 <400> 7
 aaaagtacat atttcttcaa aggaaaaaag caaaagatgt ttt 43

 <210> 8
 <211> 154
 <212> DNA
 <213> Bacillus subtilis

 <400> 8
 aaaagtacat atttcttcaa aggaaaaaag caaaagatgt ttttagctga aggaaaaatg 60
 aaaacgaaag ataaaaacag aggctgaaag ccatttttaa gcgtttttct tttcttggtg 120
 catcatttac aatacatata accgcaagga gagg 154

 <210> 9
 <211> 166
 <212> DNA
 <213> Bacillus subtilis

 <400> 9
 aaaagtacat atttcttcaa aggaaaaaag caaaagatgt ttttagctga aggaaaaatg 60
 aaaacgaaag ataaaaacag aggctgaaag ccatttttaa gcgtttttct tttcttggtg 120
 catcatttac aatacatata accgcaagga gaggaggaat cgcattg 166

 <210> 10
 <211> 10
 <212> DNA
 <213> Bacillus subtilis

 <400> 10
 ggtataatat 10

 <210> 11
 <211> 10
 <212> DNA
 <213> Bacillus subtilis

 <400> 11
 agttgtaaag 10

 <210> 12
 <211> 13
 <212> DNA

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<213> Bacillus subtilis

<220>
 <221> misc_feature
 <222> (5)..(9)
 <223> n is a, g, t or c

<400> 12
 aaaannnnnt ttt

13

<210> 13
 <211> 13
 <212> DNA
 <213> Bacillus subtilis

<220>
 <221> misc_feature
 <222> (5)..(9)
 <223> n is a, g, t or c

<400> 13
 ataannnnnt ttt

13

<210> 14
 <211> 13
 <212> DNA
 <213> Bacillus subtilis

<220>
 <221> misc_feature
 <222> (5)..(9)
 <223> n is a, t, g or c

<400> 14
 aatannnnnt att

13

<210> 15
 <211> 13
 <212> DNA
 <213> Bacillus subtilis

<220>
 <221> misc_feature
 <222> (5)..(9)
 <223> n is a, g, t or c

<400> 15
 aaaannnnnt att

13

<210> 16
 <211> 27
 <212> DNA
 <213> Bacillus subtilis

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<220>
 <221> misc_feature
 <222> (5)..(9)
 <223> n is a, g, t or c

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> n represents between 10 and 150 nucleotides that can be a, g, t or c

<220>
 <221> misc_feature
 <222> (19)..(23)
 <223> n is a, g, t or c

<400> 16
 aaaannnnnt tttnaaaann nnnntttt 27

<210> 17
 <211> 30
 <212> DNA
 <213> unknown

<220>
 <223> forward primer to amplify the ssb promoter region

<400> 17
 gcgaagcttc caaacattga cgaagagtct 30

<210> 18
 <211> 31
 <212> DNA
 <213> unknown

<220>
 <223> Reverse primer to amplify the ssb promoter region

<400> 18
 gctggatcct cggttaagca taagaaagac c 31

<210> 19
 <211> 315
 <212> DNA
 <213> unknown

<220>
 <223> PCR amplification product using Bacillus subtilis as template source and SEQ ID NO: 17 and SEQ ID NO:18 as primers

<400> 19
 gcttccaaac attgacgaag agtctaaaaa agcagttatc gagcgtttca acaacgttct 60
 gacttctaac ggtgcggaaga tcaactggaac aaaggattgg ggtaaacgctc gtcttgctta 120
 cgaaatcaac gatttccgcg acggtttcta ccaaatcgta aacgttcaat ctgacgctgc 180
 ggcagttcaa gaatttgacc gtctagctaa gatcagtgac gatatcattc gccacattgt 240
 tggttaaagaa gaagaataag caattttgaa atatataatg gtaaaagggtg gtcttttctta 300

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tgcttaaccg aggat

315

<210> 20
<211> 28
<212> DNA
<213> unknown

<220>
<223> Forward primer to amplify the rpsF promoter region

<400> 20
gcgaagcttg tgactttgag cggggcctt

28

<210> 21
<211> 622
<212> DNA
<213> unknown

<220>
<223> PCR amplification product using Bacillus subtilis as template source and SEQ ID NO:18 and SEQ ID NO:20 as primers

<400> 21
gcttgtagct ttgagcgggg cttcattcgt gctgagacag ttgcttatga ggatcttctt 60
gcgggcggcg gtatggcagg agctaaagag gcaggaaaag tccgccttga agggaaagaa 120
tatgtggtcc aagacggaga tggtattcat ttccgattta atgtatagga tgcagttgta 180
aagggacaag agctttggta taatataaaa ttgtgagtaa tagaattatt gtccttgcc 240
cattatgggc cgcttagtcc aaaaggaggt gcaaacagat gagaaagtac gaagttatgt 300
acattatccg ccaaacatt gacgaagagt ctaaaaaagc agttatcgag cgtttcaaca 360
acgttctgac ttctaacggt gcggagatca ctggaacaaa ggattgggggt aaacgtcgtc 420
ttgcttacga aatcaacgat ttccgcgacg gtttctacca aatcgtaaac gttcaatctg 480
acgctgcggc agttcaagaa ttgaccgctc tagctaagat cagtgacgat atcattcgcc 540
acattgttgt taaagaagaa gaataagcaa ttttgaaata tataatggta aaaggtggtc 600
tttcttatgc ttaaccgagg at 622

<210> 22
<211> 30
<212> DNA
<213> unknown

<220>
<223> Reverse primer to amplify the rpsF promoter region

<400> 22
gctggatcca tcttcgtcaa tgtttgggcg

30

<210> 23
<211> 331

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<212> DNA
<213> unknown

<220>
<223> PCR amplification product using *Bacillus subtilis* as template
source and SEQ ID NO:20 and SEQ ID NO:22 as primers

<400> 23
gcttgtgact ttgagcgggg cttcattcgt gctgagacag ttgcttatga ggatcttctt 60
gcgggcggcg gtatggcagg agctaaagag gcaggaaaag tccgccttga agggaaagaa 120
tatgtggtcc aagacggaga tggtattcat ttccgattta atgtatagga tgcagttgta 180
aagggacaag agcttttgga taatataaaa ttgtgagtaa tagaattatt gctccttgcc 240
cattatgggc cgcttagtcc aaaaggaggt gcaaacagat gagaaagtac gaagttatgt 300
acattatccg cccaaacatt gacgaagatg g 331

<210> 24
<211> 26
<212> DNA
<213> unknown

<220>
<223> Forward primer to amplify the ywpH promoter region

<400> 24
cccaagcttt caagctgtca atgccg 26

<210> 25
<211> 27
<212> DNA
<213> unknown

<220>
<223> Reverse primer to amplify the ywpH promoter region

<400> 25
cgcgatccg attgaacatg cgattcc 27

<210> 26
<211> 300
<212> DNA
<213> unknown

<220>
<223> PCR amplification product using *Bacillus subtilis* as template
source and SEQ ID NO:24 and SEQ ID NO:25 as primers

<400> 26
gctttcaagc tgtcaatgcc ggaaaaaaaaa ttgagctttc agtggtttgc gtgggatggc 60
tcttcctatg tgcgcatgaa tacgcaaaac tggctgacaa agcaaattctt tttccgtttt 120
ttaaaaagta catatttctt caaaggaaaa aagcaaaaga tgtttttagc tgaaggaaaa 180
atgaaaacga aagataaaaa cagaggctga aagccatttt taagcgtttt tcttttcttg 240

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ttgcatcatt tacaatacat acaaccgcaa ggagaggagg aatcgcatgt tcaatcggat 300

<210> 27
<211> 27
<212> DNA
<213> unknown

<220>
<223> amyE-1: Forward primer

<400> 27
gctctagacg aattatatgg atgtgac 27

<210> 28
<211> 30
<212> DNA
<213> unknown

<220>
<223> amyE-2: Reverse primer

<400> 28
gcctagagct cggatctcct tttccgattg 30

<210> 29
<211> 34
<212> DNA
<213> unknown

<220>
<223> Kn-1: Forward primer

<400> 29
caatctgcag tcgcatgag aatagtgaat ggac 34

<210> 30
<211> 28
<212> DNA
<213> unknown

<220>
<223> Kn-2: Reverse primer

<400> 30
cgggtacctc aaaatggat gcgttttg 28

<210> 31
<211> 269
<212> DNA
<213> unknown

<220>
<223> artificially made spoVG RBS

<400> 31
gaagatctct cgagggtacc ttgataact aatgctttta tatagggaaa aggtggtgaa 60

ctcatatgaa tcgagcttct agagagctcc catggaacta tgtgattacg aaaatatatc 120

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ccttttccac cacttgagta tacttagctc gaagatctct cgagggtacc ttgatacacc 180
 taatgctttt atatagggaa aaggtggtgc atggaactat gtgattacga aaatatatcc 240
 cttttccacc acttgagtat acttagctc 269

<210> 32
 <211> 29
 <212> DNA
 <213> unknown

<220>
 <223> spoVG1 primer

<400> 32
 gaagatctct cgagggtacc ttgatacac 29

<210> 33
 <211> 30
 <212> DNA
 <213> unknown

<220>
 <223> spoVG2 primer

<400> 33
 ctatataaaa gcattagtgt atcaaggtag 30

<210> 34
 <211> 30
 <212> DNA
 <213> unknown

<220>
 <223> spoVG3 primer

<400> 34
 ctaatgcttt tatatagggga aaaggtggtg 30

<210> 35
 <211> 30
 <212> DNA
 <213> unknown

<220>
 <223> spoVG4 primer

<400> 35
 ctcgattcat atgagttcac caccttttcc 30

<210> 36
 <211> 29
 <212> DNA
 <213> unknown

<220>
 <223> spoVG5 primer

Substitute Sequence Listing

<400> 36
gactgcagct cgagggtacc ttgatacac 29

<210> 37
<211> 41
<212> DNA
<213> unknown

<220>
<223> Gntterm-1: Forward primer

<400> 37
gcggatccag gcctaactaa ttaacctgta ttaaaaacac g 41

<210> 38
<211> 33
<212> DNA
<213> unknown

<220>
<223> Gntterm-2: Reverse primer

<400> 38
gctctagagt taaccttctg ttgtttggga tag 33

<210> 39
<211> 40
<212> DNA
<213> unknown

<220>
<223> B-toxoid3 primer

<400> 39
aactgcagag atctcatatg aagaaaaaat ttatttcatt 40

<210> 40
<211> 32
<212> DNA
<213> unknown

<220>
<223> Btoxoid2 primer

<400> 40
cgcggtacct taaatagctg ttactttgtg ag 32

<210> 41
<211> 33
<212> DNA
<213> unknown

<220>
<223> rpsF-3 primer

<400> 41
ggaattcctg caggtgactt tgagcggggc ttc 33

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<210> 42
<211> 24
<212> DNA
<213> unknown

<220>
<223> rpsF-4 and rpsF-5 primer

<400> 42
cgtactttct catatgtttg cacc 24

<210> 43
<211> 28
<212> DNA
<213> unknown

<220>
<223> amyEback-1 primer

<400> 43
cccaagcttt cgacatggat gagcgatg 28

<210> 44
<211> 28
<212> DNA
<213> unknown

<220>
<223> amyEback-2 primer

<400> 44
gcagctcgag gctccggcgc aaatgcag 28

<210> 45
<211> 29
<212> DNA
<213> unknown

<220>
<223> amyEfront-1 primer

<400> 45
cgcgagctca acaaaattct ccagtcttc 29

<210> 46
<211> 31
<212> DNA
<213> unknown

<220>
<223> amyEfront-2 primer

<400> 46
cggctagaa gtttttaatt tgtgtgtttc c 31

<210> 47

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<211> 23
 <212> DNA
 <213> unknown
 <220>
 <223> cmR-1 primer
 <400> 47
 cggaattct catgtttgac agc 23

<210> 48
 <211> 30
 <212> DNA
 <213> unknown
 <220>
 <223> cmR-2 primer
 <400> 48
 cgcgaagctt cccagtagta gggttgaggcc 30

<210> 49
 <211> 41
 <212> DNA
 <213> unknown
 <220>
 <223> Gntterm-3: Forward primer
 <400> 49
 gcggatccta cgtaactaa ttaacctgta ttaaaaacac g 41

<210> 50
 <211> 315
 <212> DNA
 <213> unknown
 <220>
 <223> upstream region of the rpsF gene, indicating the location of the
 -10 and -35 regions and AT-rich stretches further upstream, as
 well as the RBS and the start codon of the rpsF gene

<400> 50
 ctgagtgtgc aggaattatt catagtgact ttgagcgggg cttcattcgt gctgagacag 60
 ttgcttatga ggatcttctt gcgggcggcg gtatggcagg agctaaagag gcaggaaaag 120
 tccgccttga agggaaagaa tatgtggtcc aagacggaga tgttattcat ttccgattta 180
 atgtatagga tgcagttgta aagggaacaag agctttggta taatataaaa ttgtgagtaa 240
 tagaattatt gctccttgcc cattatgggc cgcttagtcc aaaaggaggt gcaaacagat 300
 ggaagttact gacgt 315

<210> 51
 <211> 312
 <212> DNA
 <213> unknown

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<220>

<223> upstream region of the ywpH gene

<400> 51

tcaagctgtc aatgccgga aaaaaattga gctttcagtg gtttgcgtgg gatggctctt	60
cctatgtgcg catgaatacg caaaactggc tgacaaagca aatctttttc cgttttttta	120
aaagtacata tttcttcaaa ggaaaaaagc aaaagatgtt tttagctgaa ggaaaaatga	180
aaacgaaaga taaaaacaga ggctgaaagc cttttttaag cgtttttctt ttcttggtgc	240
atcatttaca atacatacaa ccgcaaggag aggaggaatc gcatgttcaa tcagggtcatg	300
cttgtcggac gt	312